SUPPLEMENTARY MATERIAL

Methods and Materials

Isolates and Antifungal susceptibility testing

This was a retrospective study on *C. auris* isolates collected at Hamad Medical Corporation (HMC) in the Division of Microbiology between March 2020 and Jun 2021 during the large waves of the COVID-19 pandemics. HMC is the major provider of secondary and tertiary healthcare and has 12 hospitals, and these isolates were from 9 different tertiary healthcare institutions: mainly from Hamad General Hospital, Hazm Meberik Hospital and Cuban Hospital (Table S1). Of note, Hazm Meberik Hospital and Cuban Hospital were designated for patients with COVID-19 pneumonia particularly to those in need of admission to medical wards or intensive care units.

Patients were confirmed SARS-CoV-2 by positive real-time polymerase chain reaction (RT-PCR) assays performed at the HMC Central Laboratory or Sidra Medicine Laboratory, following standardized protocols [1]. Nasopharyngeal and/or oropharyngeal swabs were collected for PCR testing and placed in Universal Transport Medium (UTM). Aliquots of UTM were tested with real-time RT–qPCR using TaqPath COVID-19 Combo kits (Thermo Fisher Scientific) on an ABI 7500 FAST (Thermo Fisher); tested directly on the Cepheid GeneXpert system using the Xpert Xpress SARS-CoV-2 (Cepheid); or loaded directly into a Roche cobas 6800 system and assayed with a cobas SARS-CoV-2 Test (Roche). Routine blood cultures were performed using Bactec FX automated Blood culture system (BD Diagnostic, New Jersey, United States). Urine samples were cultured on cysteine lactose electrolyte-deficient agar (CLED) (Mast Diagnostics, UK). Patients were diagnosed with invasive candidemia that is defined as at least one positive blood culture and/or deep-seated candidiasis defined as positive culture obtained from sterile sites [2,3]. *Candida auris* from blood, urine, body fluid samples, as well as screening specimens (axilla, groin, nasal

swabs) were inoculated on chromogenic agar *Candida* (Oxoid, UK) and incubated at 42C for 5 days. The isolates were identified using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany) [4,5]. The antifungal susceptibility (AST) patterns were measured either by commercial system YeastOne Sensititre (TREK Diagnostic Systems, USA) or Vitek 2 (bioMerieux). AST was measured for all isolates from invasive infections (blood), and a few sporadic screening isolates; however duplicate isolates from the same patient within a week were not measured. Minimum inhibitory concentrations (MIC) were interpreted according to tentative breakpoints recommended by the US Centers of Disease control and Prevention (https://www.cdc.gov/fungal/candida-auris/recommendations.html). Patients' medical records were reviewed for travel history and antifungal therapy in the month prior to the isolation of *C. auris*.

Genome sequencing and bioinformatics analysis

For whole genome sequencing (WGS), DNA was extracted using MasterPure Yeast DNA purification kit (Lucigen Corporation, WI) and quantified using Qubit 2 fluorometer (ThermoFisher). DNA libraries were constructed with Nextera XT method (Illumina Inc, USA) and sequenced on Illumina NextSeq 550 platform with 150bp PE reads at Sidra Medicine, Qatar. FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to assess the quality of the reads, and the adaptors were trimmed by Trim Galore v0.6.0 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Trimmed reads were mapped to reference genome of *C. auris* strain B8441 (GCA_002759435.2) using Snippy v4.41 (https://github.com/tseemann/snippy). Reference strain B8441 was drug sensitive and had low tolerance (Burrack et al. 2022). Reads were aligned using BWA v7.17[6], and the

variants were called using Freebayes v1.3 (https://github.com/freebayes/freebayes) with QUAL >30 and DP >10. Variants in genes related to/associated with antifungal resistance [7,8,9] were annotated and retrieved using bcftools [10]. Variants were also visually inspected via Tablet [11]. Also, the reads were assembled *de novo* using SPAdes v.3.9.0 [12]. FastTree [13] was used to study the genetic relationships among the samples, in addition to 44 isolates published in 2021 [4], plus two unpublished duplicated isolates (CAS22, CAS35) reported in 2020. The tree was annotated by iTOL v5 [14].

Results

Variant analysis

During the in-depth variant analysis, we observed undocumented SNPs/indels in *TAC1b* (B9J08_004820) from the non-COVID-19 outbreak *C. auris* isolates reported in the early investigation [4]. In addition to substitution A640V, A583S was identified in isolates CAS20044 and CAS3357; S192N was identified in isolate CAS16, while F841del was identified in isolate CAS12, and premature codon Q612* was found in CAS29 (Table 2). Premature stop codon (W805*) was reported in CAS1, CAS15, and CAS52.

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